Draft: 5/12/98

International Dolphin Conservation Program Act (IDCPA)
RESEARCH PLAN

Marine Mammal Division
Southwest Fisheries Science Center
National Marine Fisheries Service
La Jolla, CA
May 1998

TABLE OF CONTENTS

* page numbers that appear on appendix pages refer to page numbers within individual workshop reports, not the actual page number of the research plan

ABSTRACT

This report summarizes the research plan proposed by NMFS in response to the mandates of Section 304: Research, in the International Dolphin Conservation Program Act (Public Law 105-42) for abundance surveys and studies of fishery-related stress in dolphins affected by the tuna purse-seine fishery in the eastern tropical Pacific Ocean (ETP). This report presents the objectives, format and time schedule for each of the proposed or ongoing projects, with respect to how each project will contribute to the Secretary's preliminary finding by March 31, 1999 and the final finding by December 31, 2002 regarding whether the intentional encirclement of dolphins with purse-seine nets is having a significant adverse impact on any depleted dolphin stock in the ETP.

Three abundance surveys will provide current estimates of abundance for stocks of dolphins affected by the ETP tuna purse-seine fishery with particular emphasis on the current abundance of the two depleted stocks (northeastern offshore spotted dolphin, *Stenella attenuata*, and eastern spinner dolphin, *Stenella longirostris orientalis*). Stress studies will investigate possible fishery-induced stress resulting from the process of chase and encirclement. Both approaches will attempt to estimate whether the practice of "dolphin fishing" causes significant levels of unobserved mortality or is significantly affecting population dynamics through decreased survival and/or reproduction.

Abundance surveys will be conducted during the period August-December in each of the years 1998, 1999, and 2000. Three survey vessels will be used during 1998 to provide the tightest possible confidence limits on the first abundance estimate, which will likely form the basis for the Secretary's preliminary decision in March 1999. Two survey vessels will be used in the latter two years, due to cost considerations. The surveys will include aerial photogrammetry and will collect oceanographic, acoustic, seabird and prey data in addition to dolphin abundance data in order to refine the abundance estimates.

Stress studies will include a review of current and historical literature on stress in mammals, a necropsy program, three types of analyses using historical data (cow-calf comparisons, cell stress indicators, and reproductive rates), estimation of dolphin capture frequency, and a chase/recapture experiment. The literature review will aid in identifying stress measures that may be applicable for studying stress in ETP dolphins. The necropsy program will provide various types of tissue and blood samples as a "snapshot" of stress measures in individual dolphins killed incidentally during commercial fishing operations. Cow-calf comparisons will determine whether unidentified calf mortality may be occurring in dolphin sets. The study of cell stress indicators will examine genetic material from stressed versus unstressed dolphins. The reproductive rate study will compare current and historical reproductive rates of ETP dolphins.

The capture rate study will examine methods to measure capture rate (i.e., encirclement rate) *in situ* in order to better understand the frequency with which individual dolphins actually experience chase and encirclement in the ETP. The chase/recapture experiment will examine changes in blood parameters with repeated captures of individual dolphins, to examine the level and persistence of various stress measures related to chase and encirclement.

The preliminary finding in March 1999 will likely depend almost entirely on the results from the first abundance survey. The final finding will integrate the information from all studies.

INTRODUCTION

The International Dolphin Conservation Program Act (IDCPA) was passed in August 1997 as amendments to the Marine Mammal Protection Act of 1972. A number of research projects mandated by the IDCPA are intended to determine "whether the intentional deployment on or encirclement of dolphins with purse-seine nets is having a significant adverse impact on any depleted dolphin stock in the eastern tropical Pacific Ocean". These research projects include three abundance surveys to estimate current abundance of dolphin stocks affected by the fishery and four categories of studies to evaluate whether fishery-related stress in ETP dolphins is hampering either reproduction or survival, particularly of the depleted stocks.

This report summarizes the objectives, format or structure, and proposed time schedules for the abundance and stress research projects that will be conducted in response to the directives in the IDCPA. The report is intended to inform those who may be interested in the status and progress of these research projects. A research schedule appears as Figure 1.

Greater detail on the proposed abundance surveys and stress studies can be found in the two workshop reports included as Appendices I and II in this report. Discussions of research projects presented here are summaries; greater detail can be obtained by contacting project leaders at the Southwest Fisheries Science Center (SWFSC) directly (Population Studies: Dr. Tim Gerrodette, Stress Studies: Dr. Liz Edwards, Ecology: Dr. Steve Reilly, Historical Data: Dr. Andy Dizon).

RESEARCH PROJECTS

POPULATION STUDIES

These studies will attempt to estimate the size of the adverse impact of the tuna fishery on dolphins at the population level. Studies will focus on the depleted stocks of northeastern offshore spotted and eastern spinner dolphins. Mortality in purse-seine nets represents one obvious adverse impact on dolphin populations. This impact is known because observers are on all tuna vessels over 400 tons capacity. However, the practice of catching tuna by setting on dolphins involves chasing, encircling, and confining millions of dolphins per year. Thus, it is possible that the fishery may have indirect effects on dolphin populations that go beyond direct mortality. These indirect effects may lower the reproductive rate, the survival rate, or, ultimately, population growth rate. We plan studies to estimate impacts on reproductive rates and population growth rates. Estimating survival rates for pelagic dolphins is extremely difficult.

Impacts on Reproductive Rates

Under suitable conditions, a dolphin school may be photographed from the air using high-resolution, motion-compensated cameras. Reproductive rate can be assessed as the percentage of calves present in the school. If the adverse impact of the fishery affects breeding, conception, gestation, birth or very early survival (i.e., events prior to the nursing calf stage), we would expect to see a lower percentage of calves in areas of heavy fishing, compared to areas less heavily fished. The comparison is complicated by possible

geographic (stock) differences in reproductive rates and/or timing; therefore, the rate of calf production will be measured in a number of different areas. The current rate of calf production will also be compared with the rate observed a decade ago during the Monitoring of Porpoise Stocks (MOPS) surveys. Adverse impact will be estimated by the reduction (if any) in calf production.

Most of the aerial photogrammetry will take place during the surveys for estimating abundance in 1998-2000, using NOAA's Hughes 500D helicopter. Some fixed-wing aircraft may be utilized to obtain samples in other geographic areas. Photographs will be analyzed at SWFSC.

Impacts on Population Growth Rates

The populations of northeastern offshore spotted dolphins and eastern spinner dolphins are estimated to be at about 20% of their historical population size - i.e., at 1/5 of carrying capacity. The numbers of reported mortalities due to the fishery at the present time are small fractions of population sizes, and such mortality should, therefore, have negligible effects on population growth rates. If the only adverse impact of the fishery is due to direct mortality, the depleted populations should be starting to recover. On the other hand, if the fishery has other, indirect impacts on dolphin populations, the growth rates of the dolphin populations will be reduced over what they otherwise would be. If the indirect effects are of sufficient strength, we may be able to detect the difference between the observed and expected rates of population growth.

The first step will be to obtain new estimates of population size. The IDCPA specifies that surveys to estimate dolphin abundance should occur in 1998, 1999, and 2000. The cruises will take place between late July and early December (120 sea days) of each year, primarily because that is the time of best weather, but also for comparability with the MOPS cruises of 1986-90. Estimation of abundance will be based on line-transect methods as in previous surveys although we will be using some additional newer techniques, such as passive acoustics, to improve the estimation. Because a preliminary finding is required by March, 1999, we will utilize three ships (Jordan, MacArthur and Endeavor) in 1998 to obtain more precise population estimates. The surveys in 1999 and 2000 plan to use only two vessels. Details of the survey methodology are given in Appendix I (Abundance Workshop Report) of this Research Plan.

The second step will be to use the new abundance information to estimate the current rates of population growth. The new population estimates will be combined in population models with estimates from the MOPS surveys and the time-series of relative abundance from tuna vessels. Using Bayesian methods, we will obtain probability distributions that represent our knowledge about the current growth rates for both northeastern offshore spotted and eastern spinner dolphin populations.

The third step will be to estimate the expected rates of population growth. The expected rates depend on the maximum intrinsic rates of growth (r_{max}), the current sizes of the populations relative to carrying capacity, possible time lags in the response of the populations after release from heavy mortality, and other factors of population dynamics. In constructing the population models, we will make use of historical life-history information at the SWFSC. We will also use past estimates of absolute abundance (from research vessels), the time-series of relative abundance (from tuna vessels), and estimates of the number of

dolphins killed each year since the beginning of the fishery. The output of the analysis will be probability distributions of the expected rates of population growth for both northeastern offshore spotted and eastern spinner dolphins.

The fourth step will be to estimate the adverse impact of the fishery as the difference between the expected rate and the current, observed rate for each dolphin stock. If the difference between the two rates is large, it is an indication that something is preventing dolphin populations from growing. The adverse impact of repeated chases and captures by fishermen would likely be the most parsimonious explanation of such a difference, but other explanations would be possible. A difference between expected and observed growth rates would also occur if: (1) the number of dolphins actually killed in the fishery is higher than the number reported; (2) there is substantial but unreported dolphin mortality in other fisheries in the area; (3) there has been a shift in dolphin distributions that results in an apparent decrease in dolphin abundance within the study area; and/or (4) there has been some ecological shift in the ETP ecosystem that has lowered the carrying capacity for dolphins. If there is evidence that one or more dolphin stocks is not recovering, these alternative explanations will have to be considered. Information from other parts of the IDCPA Research Plan, such as the chase/recapture experiment, necropsy samples, and ecological studies, will contribute to assessing these hypotheses.

It may also be possible to estimate the adverse impact on population growth rate in a more direct manner. Using a surrogate such as length of chase, or total time from beginning of chase to release from net, as a measure of the strength of the adverse impact, we can estimate how much this factor has effected the population growth rate. This depends on there being sufficient contrast (i.e., lack of correlation) between the direct mortality and the indirect (stress) impact to allow the effects of these two factors to be separated.

ECOLOGY STUDIES

The Ecology Program will conduct research on the physical and biological environment of the dolphin populations targeted by the IDCPA. This research will include a number of projects focusing on oceanography, dolphin prey, and the surface predator community (of which the dolphins are a component).

Oceanography

In past projects we developed methods to depict the physical habitat occupied by dolphin populations, using standard oceanographic field methods and recent multivariate statistical techniques. We found that spotted, spinner and common dolphins had characteristic, separable habitat types. All of these habitats showed some interannual variability in the ETP, and it is now regarded as essential to monitor this environmental variability to allow interpretation of dolphin sightings data. Further, the statistical depiction of oceanographic habitats allows us to make more precise abundance estimates. Therefore we plan to conduct oceanographic monitoring as part of regular daily activities on all vessels engaged in IDCPA field research.

Predator Community

The ecological basis of the ETP tuna purse seine fishery is the association of subsurface tunas with a number of surface predators, including dolphins and sea birds. Fishermen

have long used the occurrence of dolphins or birds or both to locate and capture the tunas. In past ETP dolphin assessment cruises we have recorded sea bird sightings as well as dolphin sightings in order to improve our understanding of this surface community as a whole. Considerable progress has been made. We have identified consistent patterns in dolphin-bird community structure, and tied these patterns to gradients in the environment. We plan to continue collecting bird data on the IDCPA cruises in order to check for changes since the mid-eighties in community structure. Also, because seabirds are highly visible and easily identifiable, they can be used as an indicator of the state of the community of which they are a part.

We plan to address specifically this "index" concept over the next three years in order to determine the extent to which information on seabirds can be used similarly to our use of oceanography to improve our ability to assess the status of ETP dolphin populations. By examining the bird data in conjunction with data on oceanography and shared prey (see below), we hope to shed light on the extent to which observed changes in the dolphin populations (or lack thereof, e.g. failure to show signs of recovery) may result from large-scale environmental changes such as regime shifts as opposed to the direct or indirect effects of the tuna fishery.

Prey

We plan to conduct two types of studies of dolphin prey. First, we will utilize continuous acoustic sampling devices on all ships to measure the so-called deep scattering layer, known to be comprised of fishes and cephalopods that dolphins, birds and other predators eat. Trawl samples are also planned. Further, we plan to conduct limited studies of flying fish. They are a common prey item among the surface predator community, are confined to the upper few meters of the ocean during all life stages, and are easily observed and counted. They are also tightly linked to surface water productivity and so are good organisms to use to understand the effects of oceanographic changes on the surface community. On one ship only we will conduct intermittent visual sampling (coincident with bird sightings) and nightly dipnet stations during the oceanographic station).

Sea Turtles

While sea turtles don't associate tightly with dolphins, they are distributed throughout the ETP, are part of the region's large predator community, and are incidentally taken in a number of commercial fisheries including purse-seines and long-lines. In Section 304(b)(D) the IDCPA directs NMFS to undertake or support "projects to determine the extent to which the incidental take of nontarget species, including juvenile tuna, occurs in the course of purse-seine fishing for yellowfin tuna in the eastern tropical Pacific Ocean, the geographic locations of the incidental take, and the impact of that incidental take on tuna stocks and nontarget species". All Pacific turtle species are listed as either threatened or endangered under the ESA. Little is known of their at-sea distribution or abundance, and so the IDCPA field program can make a significant contribution to NOAA's sea turtle conservation efforts for an extremely small cost. As in past ETP dolphin surveys, observers will systematically record turtle sightings on all ships. Turtle sightings are infrequent, and this has been found not to interfere with observers' dolphin monitoring duties. We also plan to conduct limited satellite tracking to measure turtle dive profiles to determine

the amount of time they spend at the surface and to allow us to estimate actual abundance as opposed to just an index of occurrence.

STRESS STUDIES

The stress research mandated under Section 304(a) of the IDCPA includes 4 general topics: a literature review, a necropsy program, historical data analyses, and a chase/recapture experiment. Each of the projects is intended to provide complementary information to the other projects and as a whole is intended to provide an overall picture of the potential effects of fishery-induced stress on ETP dolphins that can be used to guide the Secretary's final determination.

Each project is described in greater detail below. Descriptions include project objectives, integration with other studies, project structure and activities, and project time lines. The literature review is currently in progress. The other projects are in the planning stages, with the necropsy program having highest current priority in order to begin sampling from the commercial fleet as soon as possible. The historical data analyses are currently under discussion to identify appropriate research foci. The chase/recapture experiment will be a complicated field endeavor and will require considerable additional planning with respect to details, and thus is not anticipated to begin prior to 2000. The capture rate research is currently under development but should be less complicated to plan than the chase/recapture experiment so research is projected to begin during 1999.

Literature Review

Research mandated by the IDCPA requires that NMFS undertake a review of the scientific literature on stress in mammals. This review is currently in progress, and is due to be completed in September 1998. The finished product will focus on synthesizing information regarding stress in mammals as it relates to the potential impact of the tuna purse-seine fishery on dolphins in (ETP).

The review will very briefly provide background information on the ETP tuna purse-seine fishery describing its history and general aspects of dolphin-fishing operations as they have evolved over the course of time. In addition to direct mortality, several potential impacts of the ETP fishery on dolphins will be considered. These include the possible effects of acute and/or chronic stress on dolphins as a result of fisheries operations.

Background information on the concept of stress and research on stress in mammals will be provided. An extensive review of the literature on physiological stress in mammals will form the basis for a subsequent discussion of specific aspects of fisheries operations as they may be expected to affect dolphins in the ETP. Both physiological and behavioral responses to stress will be discussed. The review will include relevant research on stress effects and indications of stress on major physiological systems. Relevant research regarding effects of stressors on the autonomic nervous system (altering the functions of the cardiovascular and gastrointestinal systems, or causing the release of catecholamines from the adrenal gland for example), and the neuroendocrine system (altering the secretion of hypophyseal hormones) will be presented.

Current knowledge of stress induced pathologies and the effects of stress on suppression of immune function and disruption of reproduction will be reviewed. In addition, recent studies have been able to characterize and quantify the effects of pursuit or chase on ani-

mals, and these will help form the basis for relating literature on behavioral and physiological responses to stress in mammals to the potential influences of fisheries-induced stress on dolphins in the ETP.

Information resulting from applied research on physiological and behavioral stress responses in mammals will be combined with knowledge of cetacean behavior and physiology to examine aspects of the fishery potentially affecting dolphins. These fisheries operations include the various elements of chase (e.g. speed, duration, disruption of herd structure) and encirclement (e.g. capture, confinement). Thus, direct interactions between the fishery and dolphins, will be compared to current knowledge of mammalian responses to stress and related to the potential effects on dolphin survival.

Necropsy Program

The Necropsy Program will evaluate the pathophysiological condition of dolphins killed in the eastern tropical Pacific tuna purse-seine fishery. Collection of specimens will be accomplished by placing trained technicians on tuna purse-seine vessels to necropsy dolphins killed in fisheries interactions. Necropsy sampling will be performed in a hierarchical fashion, collecting from a prioritized list and depending upon the circumstances (number of dolphin mortalities, work space, etc.) at the time of examination. General objectives include determination of overall pathology and health/disease status of the animals.

More specific aims of the proposed Necropsy Program include examination of tissues for indications of acute and chronic stress. For example, levels of stress related hormones and catecholamines can be measured if relatively fresh blood samples are obtained. Morphological changes (e.g., hypoplasia, hyperplasia, and atrophy of certain glands and organs) documented to occur as the result of stress in mammals can be assessed. In addition, immune status and body condition of individuals will be determined. Some aspects of the project are outlined below.

These samples will be invasive collections of tissues soon after death, which can only be accomplished in this situation by necropsies on dolphins killed incidental to fishing operations (directed lethal take is not considered permissible). The samples will provide a picture of the animal's "stress profile" at a given instant in time, although some measures will provide indications of long-term effects while others will indicate more immediate effects. This project thus complements the information that will be collected during the chase/recapture experiment, which will provide serial blood samples from individual animals but will not provide any data that would require invasive sampling of tissues.

A Necropsy Sampling Program planning report providing details of proposed sampling protocols and subsequent research analyses will be completed in June 1998. The report will include an outline of the types of sampling and equipment that will be necessary to perform complete necropsies of dolphins in the ETP tuna purse-seine fishery. Hierarchical levels for prioritizing collection of necropsy samples will be defined. The report will also include an overview of research analyses to be used for investigating stress factors and health and disease status in these animals.

A planning meeting will be convened by the SWFSC to discuss details and logistical aspects of necropsy sampling, but scheduling of this meeting awaits assurance from the fleet and from foreign governments that the work can take place aboard their vessels.

Specimen Collection. Specimens will have to be collected in a rigorous and systematic manner that is not possible for tuna vessel observers under current circumstances (although certain observer-collected specimens may be used to augment specimens collected for the necropsy program). It will be necessary to have trained technicians or graduate students with some background, or program of study, in pathology to collect samples from specimens. To optimize the amount of information gathered from any single animal, multiple samples must be collected and the procedures could take many hours for a single postmortem dolphin.

The desired sample size is a minimum of 150 animals per year. To that end, it is anticipated that at least 8 trained necropsy technicians will be required per year. Each of these technicians could make 2-3 trips in one year. These plans are based on recent information indicating that the current average mortality in the ETP tuna purse-seine fishery is 8 dolphins per vessel per trip.

Collected specimens will be processed for histology and other preparations necessary for appropriate analyses. Samples to be collected include the following:

- heart	- thyroid gland	- brain	- bone
- adrenal glands	- spleen	- pituitary gland	- teeth
- lung	- thymus	- tonsils	- blood
- kidney	- lymph nodes	- muscle	- parasites
- liver	- stomach	- blubber/skin	
- pancreas	- intestines	- reproductive organs	

Major Courses of Examination.

1) Examination of pathology samples for indications of physiological stress. Careful collection and examination of post-mortem specimens will allow histological observation of sublethal effects of fisheries activities on the heart and heart muscle, adrenal glands, the liver and other major organs. Tissues will be examined for evidence of tissue injuries and morphological changes associated with stress (e. g. those resulting from short-term repeated exposure to high levels of catecholamines). The time sequence of injurious events is important and histological evidence of healing, if present, could provide some indication whether observed lesions are several days old or have occurred even more recently.

2) Determination of overall pathology and health/disease status of the animals. Collected specimens will also be used to provide an overall health assessment of individual to gather data on the states of health and disease in these dolphin populations. Such information is necessary for deriving conclusions regarding the effects of stress on these animals.

3) Evaluation of overall immune status of individuals. Analysis of relatively fresh blood samples can be used for complete immune assays. Examination of spleen, thymus and lymph tissue (and possibly tonsils and additional lymphoid tissues) can be used to investigate the immune status of individual dolphins collected from the fishery.

4) Estimation of general physiological condition. Body condition indices, body composition, and lipids analysis can be used in conjunction with pathology data to provide information on the physiological condition of individuals. Data on body composition and blubber depth will be useful for assessing the general health and nutritional condition of

these dolphins. These analyses can also yield information on nutritional status and can be integrated to determine energy budgets.

5) Examination of muscle tissues. Chase is a significant part of purse-seine capture of these dolphins, and the length of the chase may be a factor in the stress response. Over exerted muscle does not heal well. Examination of muscle tissues, muscle fiber typing, testing for lactic acid and creatine kinase levels, and measuring blood gases may be important for assessment of the degree and effects of muscle and respiratory exertion.

6) Detection of corticosteroid levels in cetacean blubber samples may provide a means of investigating the stress response in these animals. It will be necessary to obtain baseline information on all steroids in cetacean blubber. It will be useful to look at how all the steroids interact simultaneously. Because we do not know the dynamics of steroids in the blubber, this may require assessment of metabolic turnover rate by using an exogenous (labeled) steroid in a captive dolphin and measuring diffusion time.

Chase/Recapture Experiment

The objective for this project is to measure dynamic stress effects in blood chemistry parameters of individual dolphins over a several-week period in which the individuals are recaptured several times. These series of parameters will indicate whether stress-related effects occur in the animals' blood chemistry and if so, whether the effects are transient or cumulative. This study will provide measurements from living dolphins that will complement the tissue samples taken from freshly-dead dolphins during the necropsies.

This experiment will require the services of a chartered (dedicated) ETP tuna purse-seiner for 30-60 days in addition to those of a research vessel. The seiner will be employed to chase and encircle dolphin schools in the usual manner. The scientific research vessel will provide housing for scientists and equipment during the experiment.

During the first encirclement, scientists will attach radio and/or satellite tags to as many dolphins as possible (probably 3-5) and rototags to as many others as possible. Blood samples will be taken from the fluke vein of each radio/satellite tagged dolphins and from the fluke vein of as many rototagged animals as possible. The dolphins will be released and the radio/satellite tagged animals tracked during a recovery period of several (probably 2-5) days. The radio/satellite tagged animals will be recaptured, resampled for blood parameters, and released again. During each set, as many dolphins as possible will also be rototagged, and sampled for blood if possible. The rototagged animals will provide time-zero measures for blood parameters from each set, and may also provide some information about school fidelity if rototagged animals are re-captured during subsequent sets.

As with the life history study the concept of this experiment is simple but its execution will require extensive planning. Also, it is possible that specific permits may be required; this issue will be explored during planning meetings to be held over the next two years. Thus, the experiment is tentatively scheduled for calendar year 2000 in order to provide sufficient planning time prior to initiating the experiment.

Further details of the blood parameters proposed for sampling and other details related to this experiment can be found in the Dolphin Stress Workshop Report (Appendix II of this Research Plan).

Capture Rate Estimation

A related project will investigate capture frequency *in situ* for individual dolphins. A central question related to potential fishery-related stress effects in ETP dolphins is the number of times individual animals actually experience the "dolphin fishing" procedure. Estimates derived from combining research vessel and tuna vessel observer data indicate a potentially wide range of capture rates for individual dolphins, depending upon the frequency with which they associate with dolphin schools of various sizes . Large schools (over 1000 animals) appear to experience chase and capture as often as once a week while small schools (less than 200 animals) appear to experience chase and capture less than once or twice per year. The central unanswered question is school fidelity, i.e., how much time do individual dolphins spend in schools of particular sizes? If all dolphins are equally "unfaithful", then the simple average of 8 sets per year per animal may apply to all or at least most dolphins. If dolphins are completely "faithful" to schools of a given size, then the capture rate for individual animals could vary from less than 1 to over 50 captures per year. Obviously, the estimated potential for stress varies considerably for individual dolphins depending upon the assumptions made about school fidelity.

The objective for the Capture Rate estimation project is to determine how to measure that rate *in situ*, and then to do so. Investigation of various potential measurement methods is currently underway and is expected to be completed during calendar year 1998. Once the most appropriate method is identified, protocols can be designed to initiate measurements *in situ.*

Historical Data Analyses

The objective for this research focus is to explore new methodologies or approaches that will employ the large number of existing ETP samples stored at the SWFSC to examine the problem of adverse fishery impacts. Most of these samples were collected between approximately 1975 and 1990 when the dolphin fishery made about 10,000 sets per year and killed approximately 20,000-130,000 dolphins per year. Several thousand samples exist for each year, including reproductive organs, teeth, blood stains, and skin.

Between the time when the samples were collected and the present, many new molecular biological techniques have become routine, e.g., extracting DNA from blood-stained observers records and from formalin preserved samples. In addition, the SWFSC now has a fully equipped, modern molecular biology laboratory. These improvements in laboratory capabilities make new analyses possible that could not be accomplished earlier.

Three lines of investigation will be explored, two involving molecular approaches and a third investigating reproductive rates.

Cow-Calf Comparisons. The first study will take a comprehensive look at kill composition of individual sets to determine what the available samples reveal about the effect of chase and encirclement on the cow-calf bond. This is conceptually simple, but a variety of sample biases have to be dealt with. At its simplest, one would expect that for every lactating female killed, a calf should also be observed killed and be enumerated. If not, the kill count is biased downwards, and more importantly, the potentially serious impact of chase and encirclement on the cow-calf bond is being ignored. Molecular techniques will be used to ascertain whether existing small kill samples that comprise both cow and calves

are indeed genetically related cow-calf pairs or are simply a "random" sample of the cows and calves of the encircled school. Demonstrating the latter, of course, would suggest that dolphin sets might separate cows and their calves. If the separated calves cannot not survive without their mothers then calf mortality rates may be higher than that observed directly. Planning for this project is in progress, with work expected to begin in July 1998.

Cell Stress Indicators. The study will focus on the feasibility of observing the effects of stress at the cellular level, on genetic material. For instance, there are unique, single copy mammalian genes which produce extracellular copies in response to cellular stress. Also, concentrations increase of messenger RNA of various receptors that are indicative of neuroimmunological stress. The increased concentrations of these genes may be detectable in total cellular DNA extracted from skin. The SWFSC tissue archive has samples from both stressed animals and animals biopsied while bow riding (i.e.,while presumably unstressed or at least considerably less stressed). We will examine these samples to determine whether we can detect relative increases of these indicators in stressed (fishery killed) vs. less stressed (bow-riding) animals. While we recognize that behavioral stress may not induce these responses in skin. We also recognize that even if increased cellular components indicative of cellular stress are detected, connecting this causally to population-level effects or even individual-level effects such as decreased reproductive potential will be difficult. However, the potential advantages of identifying a measure that can be assessed in samples from presumably unstressed as well as assumed stressed animals are worth the effort. Planning for this project is also in progress, with work expected to begin in July 1998.

Reproductive Rates Comparison. The third study will focus on comparing present and past reproductive rates. Although since 1990 observed mortality rates have decreased to less than 0.2% of each stock, the number of sets involving chase and encirclement has decreased by only about 20% (7,000-8,000 sets/year). If chase and encirclement are highly stressful, the expected increase in reproduction predicted from the decrease in mortality might not be realized. While this project is conceptually simply, implementation will be complicated and sample sizes for determination of current reproductive rates may not be adequate. Implementation will be complicated because collecting current data will required reinstating the biological sampling program that was discontinued in 1990. Observers will have to be trained to collect the required material, and tuna fleet cooperation will be required for sample collection, storage, and transport. Sample sizes may not be adequate because so few animals currently comprise the observed kill. These considerations will require further investigation before it can be determined whether this line of inquiry is likely to produce significant results. Thus, research in this area will likely not begin until mid-1999, if at all.

The feasibility of each of these three projects will be considered and some preliminary work will be done during the remainder of calendar year 1998. The identification of cell stress indicators can be accomplished with samples on hand. While the other two projects (the cow-calf comparison and the reproductive vital rates studies) can make use of existing samples archived at the SWFSC, the success of each would be materially enhanced if a biological specimen sampling program can be re-established. Whether a biological sam-

pling program can be re-implemented and whether sufficient numbers of samples can be collected is under investigation but is unknown at this time.

SUMMARY

Three general categories of research related to the mandates of the IDCPA have been identified and are in various stages of planning or progress. These categories include 1) population studies, 2) ecology studies, and 3) stress studies. The majority of the projects are still in the planning stage but data collection should be initiated for most or all projects by the end of fiscal year 1998. This report summarizes the research projects proposed or in progress within each category. The schedule for various research projects appears as Figure 1. Reports from completed workshops on abundance survey and stress study planning are attached as appendices.

Figure 1. IDCPA Research Schedule

APPENDIX I: Abundance Workshop Report

This portion of the IDCPA Research Plan is contained in a separate file.

APPENDIX II: Stress Workshop Report

This portion of the IDCPA Research Plan is contained in a separate file.